REMARKS

Claims 55 to 90 are pending in this application. Claims 55, 56, 63, 65, 67, 69, 71, 73, 78, 79, 81 and 82 are under examination and claims 57-62, 64, 66, 68, 70, 72, 74-77, 80 and 83-90 have been withdrawn because they are directed to a non-elected invention or species. In view of their withdrawal from consideration, claims 58 to 62, 75, 80 and 83 to 90, directed to a non-elected invention, have been canceled without prejudice. Applicant reserves the right to prosecute the subject matter of the canceled claims in the present application and/or one or more related applications. Claims 76 to 78 have been amended to delete dependency from a canceled claim. New claims 91 to 95 have been added to the application. Support for the new claims can be found in the specification as originally filed, see, *e.g.*, the specification at page 21, lines 8-10. Thus, the new claims do not constitute new matter.

Accordingly, after entry of the present Amendment, claims 55 to 57, 63 to 74, 76 to 79, 81, 82 and 91 to 95 will be pending in the present application.

Information Disclosure Statement

Applicant notes that references C20, C21, C22, C23, C66, C67, C68 and C69 were crossed out on the revised PTO-1449 Forms filed January 9, 2007. Applicant is submitting herewith a Supplemental Information Disclosure Statement with a revised PTO-1449 that includes the dates for the references previously cited as C20, C21, C22, C23, C66, C67, C68 and C69 (resubmitted as references C82, C83, C84, C85, C98, C99, C100 and C101). Applicant respectfully requests that the Examiner consider these references and the other references listed on the revised PTO 1449 Form submitted herewith.

1. The Rejections under 35 U.S.C. § 103(a) Should be Withdrawn

A. Tocchini-Valentini in Combination with Gontarek Does Not Render The Claims Obvious.

Claims 55-56, 65, 67, 71 and 73 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Tocchini-Valentini et al., International Publication No. WO 01/92463 ("Tocchini-Valentini"), in combination with Gontarek, International Publication No. WO 00/67580 ("Gontarek"). In particular, the Examiner alleges that Tocchini-Valentini teaches methods of monitoring tRNA splicing endonuclease activity on various target

molecules and Gontarek teaches methods or assays for screening for a compound that modulates splicing reactions. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art to screen for compounds that modulate the activity of animalia tRNA splicing endonuclease because Tocchini-Valentini teaches the tools to execute such a screen, including animalia tRNA splicing endonuclease, and Gontarek teaches that tRNA splicing endonuclease reactions are useful for screening for compounds that inhibit the RNA splicing mechanism (see May 12, 2009 Office Action, p. 5). For the reasons below, the rejection should be withdrawn.

A finding of obviousness requires that "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. §103(a). In its recent decision addressing the issue of obviousness, *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 USPQ2d 1385 (2007), the Supreme Court stated that the following factors set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966) still control an obviousness inquiry: (1) the scope and content of the prior art; (2) the differences between the prior art and the claimed invention; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness. *KSR*, 127 S.Ct. at 1734, 82 USPQ2d at 1388 quoting *Graham*, 383 U.S. at 17-18, 14 USPQ at 467.

The *KSR* Court rejected a rigid application of the "teaching, suggestion, or motivation" test previously applied by the Court of Appeals for the Federal Circuit. *KSR*, 127 S. Ct. at 1739 USPQ2d at 1395. However, the Supreme Court affirmed that it is "important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does . . . because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." *KSR*, S.Ct. at 1741, 82 USPQ2d at 1396. Thus, consistent with the principles enunciated in *KSR*, a *prima facie* case of obviousness can only be established by showing a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference and to carry out the modification with a reasonable expectation of success, viewed in light of the prior art.

Thus, the principles set forth in *Graham*—which are still good law post-KSR—require that both the suggestion and the expectation of success must be found in the prior art,

and not derived from knowledge gained from the applicant's disclosure.

After the *KSR* decision, the Board of Patent Appeals and Interferences has continued to shape the contours of the obviousness inquiry. The Supreme Court in *KSR* stated that "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 127 S.Ct. at 1741, 82 USPQ2d at 1389. Following *KSR*, the Board stated that "[i]t is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *Ex Parte El-Naggar*, WL 2814131 at *3 (BPAI 2007) (citing *In re Hedges*, 783 F.2d 1038, 1041 (Fed. Cir. 1986) (quoting *In re Wesslau*, 353 F.2d 238, 241 (C.C.P.A. 1965))).

As acknowledged by the Examiner, Tocchini-Valentini does not teach or suggest a method that includes "assaying for a compound that can reduce (or inhibit) RNA splicing" as recited in claims 56, 67 and 73 (see May 12, 2009 Office Action at p. 4). Rather, Tocchini-Valentini describes utilizing the characteristics of the first step of the tRNA splicing reaction (*i.e.*, cleavage of the pre-tRNA substrate), particularly the specificity of cleavage, to develop a *method of cleaving a target RNA molecule* that has a bulge-helix-bulge conformation by exposing said RNA to a eukaryotic or archael tRNA splicing endonuclease. Tocchini-Valentini describes the use of the RNA cleavage method for the removal of unwanted RNA molecules from cells (see p. 9, paragraph [0031]), the detection of the presence of particular types of RNAs (see p. 9, paragraph [0030]), the cleavage of a target RNA into defined ends with a 2',3' cyclic phosphate capable of being ligated (see p. 8, 9, paragraph [0029]), as well as the degradation of particular targeted RNAs (see p. 9, paragraph [0029]). There is no teaching or suggestion in Tocchini-Valentini that tRNA splicing endonuclease may be used to screen for compounds that modulate the activity of animalia tRNA splicing endonuclease. Moreover, there is no teaching or suggestion in Tocchini-Valentini of utilizing in any method a nucleic acid comprising a tRNA intron within the *mature domain* of a precursor tRNA (as recited in claims 91 to 95), which is considered the natural substrate of eukaryal tRNA splicing endonuclease (see specification p. 3, lines 2-4). Thus, Tocchini-Valentini does not teach or suggest the claimed methods.

The deficiencies in Tocchini-Valentini are not cured by Gontarek. Contrary to the Examiner's contention, Gontarek does not teach that tRNA splicing endonuclease reactions are useful for screening for compounds that inhibit the tRNA splicing mechanism. Gontarek

relates to methods of screening for compounds that modulate *pre-mRNA* splicing. Gontarek describes "a method of screening for a compound modulating a pathogen or non-pathogen *splicing reaction* comprising the steps of contacting a *splicing reaction mix* with a candidate compound and identifying a compound modulating the splicing reaction." (see p. 2, lines 7-10; emphasis added). According to Gontarek, the definition of a "splicing reaction" is "any or all steps in the maturation of a eukaryotic *pre-mRNA*, including but not limited to lariat formation, branchpoint formation, spliceosome formation (or any aggregation of splicing factors or proteins on an *mRNA*, *pre-mRNA* or polynucleotide splicing signal) ... or protein bound to any RNA sequence that is, or is derived from, a *pre-mRNA* any sequence [sic]." (see p. 9, lines 26-31; emphasis added). In addition, "splicing reaction mix (es)" is defined as "a composition that allows a *pre-mRNA* to undergo a splicing reaction." (see p. 9, lines 32-33; emphasis added). Gontarek also describes "a method of [sic] wherein the splicing reaction comprises at least one component of a pathogen or non-pathogen *spliceosome*." (see p.12, lines 16-17; emphasis added). It is well-known in the art that "spliceosome" refers to a complex of RNA and protein subunits that remove the non-coding introns from unprocessed *mRNA* (Stryer, 1999, Chapter 33: RNA Synthesis and Splicing *in* Biochemistry ("Stryer"); see p. 862-864).

In contrast, the pending claims of the present application are directed to a method for identifying a compound that modulates animalia *tRNA splicing endonuclease* activity. Thus, the subject matter of the pending claims is distinguished from the subject matter of Gontarek by providing a specific target for the screening assay that is a component of the *tRNA*, not mRNA, splicing machinery, and which is tRNA splicing endonuclease.

There are fundamental differences between mRNA splicing and tRNA splicing that would not suggest to one of ordinary skill in the art to substitute one splicing pathway for another. Although both mRNA splicing and tRNA splicing involve the removal of introns, the mechanism of action for the removal of introns from pre-mRNA is very different from the removal of introns from pre-tRNA (Abelson *et al.*, 1998, J Biol Chem., 273:12685-688 ("Abelson"); see p. 12685, col. 1, first paragraph). It is well known in the art that the substrates, mechanism of action and players involved in the mRNA and tRNA splicing pathways are completely different.

First, the substrate of the mRNA splicing reaction is an intron in a pre-mRNA molecule, which has a *different role* than a pre-tRNA molecule. mRNA, or messenger RNA, is the *template for protein synthesis* and transfers genetic information from the nucleus to the

ribosomes, which are the sites of protein synthesis in the cytoplasm (Stryer, 1999, Chapter 5: Flow of Genetic Information, *in* Biochemistry; see p. 96, lines 19-22). tRNA, or transfer RNA, is the *adaptor molecule in protein synthesis* and its role is to carry specific amino acids in an activated form to the ribosome for polypeptide formation during translation (Stryer, 1999, Chapter 34: Protein Synthesis *in* Biochemistry; see p. 875-876). Moreover, the *identifying features* of the introns and their *recognition by the splicing machinery* are different between mRNA molecules and tRNA molecules in eukaryotes. mRNA introns are between 50 and 10,000 nucleotides in length and have a common structural motif, defined by a sequence that begins with the nucleotides GU at the 5' splice site and ends with the nucleotides AG at the 3 splice site, as well as an internal site located between 20 and 50 nucleotides upstream of the 3' splice site called the branch site (see Stryer, p. 860-861). The sequences at the 5' splice site and the 3' splice site, as well as the branch site, are recognized by the splicing machinery (described below) and thus play an important role in the splicing of the intron (see Stryer, p. 862-864). In contrast, tRNA introns are small (14 to 60 nucleotides in length), are located in the same position (one base to the 3' side of the anticodon in the tRNA molecule) and have no sequence conservation around the splice sites (Trotta and Abelson, 1999, tRNA Splicing: An RNA World Add-On or an Ancient Reaction? In RNA World II, Tom Cech, Ray Gesteland and John Atkins (eds.), Cold Spring Harbor Laboratory Press ("Trotta"); see p. 561, second paragraph and p. 570, second paragraph). However, the tRNA introns have a conserved base pair (the A-I base pair) between a base of the 5' exon immediately following the anticodon stem and a base in the single-stranded loop of the intron that is required for cleavage of the 3' splice site (see Trotta p. 562, first paragraph). tRNA introns are recognized either by reference to the mature domain of the tRNA molecule, whereby the tRNA splicing endonuclease (further described below) recognizes the mature domain and measures the conserved distance to the splice sites (see Trotta, p. 570, second paragraph), or by reference to the bulge-helix-bulge structure (see Trotta p. 579, first paragraph).

Second, the mechanism of action of mRNA splicing is significantly different from the mechanism of action of tRNA splicing. Pre-mRNA is spliced by the spliceosome, which is a large RNA-protein complex comprising small nuclear ribonucleoproteins (snRNPs) (see Stryer, p. 862-863). The spliceosome excises the intron and ligates the exons of pre-mRNA through two transesterification reactions, releasing the intron in the form of a lariat (see Stryer, p. 861-862). In contrast, pre-tRNA is spliced by three protein enzymes, in three

distinct steps (see Abelson; p. 12685, col. 2, second paragraph). In the first step, the tRNA endonuclease cleaves the pre-tRNA substrate either in reference to the mature domain of the substrate or by recognizing the bulge-helix-bulge structure of the substrate. In the second step, the pre-tRNA is ligated by tRNA ligase, and in the third and last step of the splicing reaction, the 2'-phosphate generated by the ligase reaction is removed from the spliced tRNA by 2' phosphotransferase. Thus, given the differences between mRNA splicing and tRNA splicing, one of ordinary skill in the art would *not* substitute tRNA splicing for mRNA splicing.

Moreover, contrary to the Examiner's allegations, a person of ordinary skill in the art would *not* have been motivated to screen for compounds that modulate the activity of animalia tRNA splicing endonuclease because of any teaching by Gontarek regarding the usefulness of screening for compounds that are inhibitors of the mRNA splicing machinery (see May 12, 2009 Office Action, p. 5). Neither Tocchini-Valentini nor Gontarek provide any indication that an *animalia tRNA splicing endonuclease* might be a suitable drug target, in particular a suitable target to identify an anti-proliferative drug. Tocchini-Valentini states that "[a]ccuracy in tRNA splicing is essential for the formation of functional tRNAs, and hence for cell viability." (see p. 13, paragraph [0047]). Given the importance of the tRNA splicing endonuclease for cell viability, a person of ordinary skill in the art would *not* have been motivated to consider animalia tRNA splicing endonuclease as a potential drug target in animals, *e.g.*, humans. One of ordinary skill in the art would have expected that a compound that modulates (in particular, inhibits) the activity of an animalia tRNA splicing endonuclease would affect cell viability, and therefore, one of ordinary skill in the art would not have been motivated to screen for compounds that modulate (in particular, inhibit) the activity of an animalia tRNA splicing endonuclease.

In view of the foregoing, the rejection of claims 55-56, 65, 67, 71 and 73 under 35 U.S.C. 103(a) should be withdrawn.

B. Tocchini-Valentini in Combination With Gontarek in View of Marras Does Not Render the Claims Obvious.

Claims 63, 69 and 73 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Tocchini-Valentini, in combination with Gontarek, as applied to claim 55 and further in view of Marras *et al.*, (*Nucleic Acids Research*, 2002, 30: 1-8 ("Marras")). In

particular, the Examiner alleges that Tocchini-Valentini teaches methods of monitoring tRNA splicing endonuclease activity using various nucleic acid substrates and Marras teaches labeling of nucleic acids with a fluorophore and a quencher in methods of nucleic acid detection. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art to label a substrate for tRNA splicing endonuclease with a fluorophore and a quencher because Tocchini-Valentini teaches nucleic acids cleaved by tRNA splicing endonuclease and Marras teaches nucleic acid detection using quenchers in combination with fluorophores to allow for the generation of a fluorescent signal from an efficient energy transfer (see May 12, 2009 Office Action, p. 6 and 7). For the reasons below, the rejection should be withdrawn.

The deficiencies of Tocchini-Valentini and Gontarek discussed above are not cured by Marras. Marras describes the efficiency of quenching of several fluorophores by different quenchers in order to *design oligonucleotide probes* for homogeneous *hybridization assays* (see abstract; see also p. 2, col. 1, third paragraph and p. 6, col. 2, second paragraph). Marras describes "two complementary oligodeoxyribonucleotides that would bring a fluorophore close to a quencher when the oligodeoxyribonucleotides hybridized to each other." (see p. 3, col. 1, third paragraph). Marras also describes the importance of several factors in designing oligonucleotide probes, including the optimal difference in length between a quencher-labeled oligodeoxyribonucleotide and a fluorophore-labeled oligodeoxyribonucleotide (see p. 3, col. 2, second paragraph), the affinity between the fluorophore and the quencher (see p. 4, col. 2, second paragraph) and the possibility of quenching by nucleotides (see p. 6, col. 1, second paragraph).

In contrast, the pending claims in the present application describe a nucleic acid *substrate* for tRNA splicing endonuclease, wherein the substrate comprises a *tRNA intron* within a *bulge-helix-bulge structure* or a *mature domain* of a precursor tRNA. The claims are thus not directed to oligonucleotide probes or hybridization assays. Marras does *not* teach or even suggest a nucleic acid substrate comprising a tRNA intron within a bulge-helix-bulge structure or a mature domain of a precursor tRNA (as in Claim 55) that is labeled at the 5' end with a fluorophore and at the 3'end with a quencher, or vice-versa (as in claim 63) or that is labeled at the 5' end with a fluorescent donor moiety and at the 3'end with a fluorescent acceptor moiety, or vice-versa (as in claim 69). Moreover, there is no teaching or suggestion in Marras that labeling a nucleic acid substrate as in claim 69 would allow the detection of the *amount of substrate cleaved* and subsequently, the *identification of a compound* that

modulates enzymatic activity, much less reduce tRNA splicing endonuclease activity, by measuring the fluorescence of the substrate, as described in claim 73. Thus, the subject matter of the claims is distinguished from the subject matter of Marras. Therefore, one of ordinary skill in the art would *not* have been motivated to label Tocchini-Valentini's nucleic acids with Marras' fluorophore and quencher to detect cleaved tRNA and identify modulators of tRNA splicing endonuclease activity.

In view of the foregoing, the rejection of claims 63, 69 and 73 under 35 U.S.C. 103(a) should be withdrawn.

C. Tocchini-Valentini in Combination with Gontarek in View of Herrenknecht Does Not Render the Claims Obvious.

Claims 78, 79, 81 and 82 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Tocchini-Valentini, in combination with Gontarek, as applied to claim 55 and further in view of Herrenknecht (*Nucleic Acids Research*, 1988, 16: 7713-7714). The Examiner alleges that Herrenknecht teaches an extract containing human tRNA splicing endonuclease in the method of *in vitro* pre-tRNA splicing. The Examiner concludes that it would have been obvious to a person of ordinary skill in the art to substitute the mouse tRNA splicing endonuclease taught by Tocchini-Valentini with the human tRNA splicing endonuclease taught by Herrenknecht to arrive at the invention in the instant application (see May 12, 2009 Office Action, p. 8). For the reasons below, the rejection should be withdrawn.

The deficiencies of Tocchini-Valentini and Gontarek discussed above are not cured by Herrenknecht. Herrenknecht describes human leukaemia cell nuclear extracts with splicing endonuclease and ligase activity. Herrenknecht does not teach or suggest screening for compounds that modulate human tRNA splicing endonuclease. Herrenknecht does not provide *any* indication that a *human tRNA splicing endonuclease* might be a suitable drug target, in particular a suitable target to identify an anti-proliferative drug. In fact, as explained hereinabove, a person of ordinary skill in the art would *not* have been motivated to consider tRNA splicing endonuclease as a potential drug target in animals, such as humans, because of the importance of the endonuclease for cell viability. Therefore, one of ordinary skill in the art would *not* have been motivated to substitute the mouse tRNA endonuclease with the human tRNA endonuclease to screen for compounds that modulate, and in particular, *inhibit*, the activity of a human tRNA splicing endonuclease.

In view of the foregoing, the rejection of claims 78, 79, 81 and 82 under 35 U.S.C. 103(a) should be withdrawn.

2. The Double-Patenting Rejection should be held in abeyance

Claims 55, 56, 58, 63, 65, 67, 69, 71, 73, 75, 78, 79, 81 and 82 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 42-64 of co-pending Application No. 10/551,304. Claims 55, 56, 58, 63, 65, 67, 69, 71, 73, 75, 78, 79, 81 and 82 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 26-48 of co-pending Application No. 10/551,300.

These are *provisional* obviousness-type double patenting rejections because the conflicting claims have not in fact been patented. Accordingly, Applicant respectfully requests that these rejections be held in abeyance until such time as there is allowable subject matter.

CONCLUSION

Applicant believes that the present claims meet all of the requirements for patentability. Consideration and entry of the amendments and remarks made herein into the file history of the present application are respectfully requested. The Examiner is invited to contact the undersigned if any issues remain.

Respectfully submitted,

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